

Application of Sol Gel Technique for Glucose Oxidase Immobilization in Biosensor Application

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Abstract

A sol gel based biosensor using 3-glycidoxypopyl dimethylethoxysilane, as a silane agent was developed as a novel method for biosensor enzyme immobilization. The key materials applied were tetra methyl orthosilicate (TMOS), and the cross link agent, 3-Glycidoxypopyl dimethylethoxysilane, (GDP). Three compositions with different amount of GDP and glucose oxidase were experimented with the amount of TMOS was kept constant. The first coating consisted of 2mg of glucose oxidase and 20 μ l of GDP. The second trial involved higher GDP composition which is 100 μ l. The final trial applied higher content of glucose oxidase which is 12 mg with 20 μ l of GDP. The result showed that sensors with high composition of crosslink agent coating was capable to exhibit reliable glucose detection. The crosslink agent insufficiency in the first composition failed to provide good attachment for the enzyme on the electrode. Thus, the test was halted after few readings due to the inability of the sensor to detect glucose increment. For the high amount glucose oxidase composition, failure happened due to the deficiency of GDP to retain the enzyme thus contributed to the glucose oxidase leaching. We conclude that adequate amount of crosslink agent is vital for a sol gel based biosensor to function successfully.

Keywords: biosensor, glucose sensor, immobilization technique, sol gel technique

1.0 Introduction

The field of biosensors had been given to birth by the first successful work on glucose sensor by Clark and Lyons in 1962 [1]. Since then, the main issue of creating reliable biosensors lies on the immobilization technique. The immobilization techniques could be generally categorized as adsorption, microencapsulation, entrapment, cross linking, covalent bonding and conducting polymers [1-6].

Sol gel technique can be categorized as combinations of both microencapsulation and cross linking methods. Basically, this technique applies wet chemistry reactions and the inorganic polymerization of molecular precursors [7].

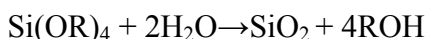
It involves low molecular weight metal alkoxide precursors $M(OR)_z$, where R is an alkyl groups i.e. CH_3- and CH_3CH_2- groups [7]. Examples of these precursors are tetramethoxysilane (TMOS; $Si(OCH_3)_4$) or tetraethoxysilane (TEOS; $Si(OC_2H_5)_4$) [8]. These precursors can be mixed at molecular level and multi component material will be

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formed at low temperature i.e. room temperature [3, 7, 9]. The oxide network is formed by hydrolysis and condensation process of the precursors as follows [7]:



The whole the interaction could be written as:



Hydrolysis results in the formation of silanol groups (Si–OH). These silanol moieties react further to form siloxane (Si–O–Si) polymers in a condensation reaction. This occurs at localized region, which would forms colloidal particle later, named as sol. When the network is formed between particles, a gel like solution would be formed, which enables it to encapsulate the enzyme due to the porous property of the gel [10].

The preparation of sol–gel system is highly influenced by few parameters (i.e., alkoxide precursor, water: Si molar ratio, pH, solvent, catalyst, temperature). These parameters affect the rates of hydrolysis and condensation and for this reason; it would allow the control of the nanostructure and microstructure of the final material [10].

This control mechanism is essential to obtain a right stability between non-leaching of the entrapped enzyme and its accessibility to the target analyte i.e. glucose. The amount of leaching will depends on the relative size of the pores that will retain the enzyme [10, 11]. In the past, usual sol gels procedures are often involve extreme pH and/or a high alcohol content that may be harmful to the activity of the enzyme [10]. Thus in this article, a novel procedure for glucose oxidase immobilization in biosensor using milder sol gel technique is being developed.

2.0 Materials and Methods

2.1 Construction of Sensors

The sensors were constructed based on a hydrogen peroxide sensor where uninsulated platinum wire (Goodfellow®) was passed along a stainless steel tube and fixed in the tube with epoxy resin (Promatech Ltd.). The tip of tube and then was polished and cleaned carefully before negative charged Nafion film was deposited as the inner membrane. The electrode tip was left to dry for an hour.

Sol gel method using three different compositions was carried out. Overall process involved division of the reaction agents and mixed in two parts. Part A consisted of Tetramethyl orthosilicate (TMOS) which was mixed with Phosphate buffer saline (PBS) and the silane agent, 3-Glycidoxypopyl dimethylethoxysilane, (GDP). Part B consisted of GOD and bovine serum albumin (BSA) diluted in PBS. Part A and Part B were thoroughly mixed.

The first batch of experiment utilized 2 mg of GOx and the amount of crosslink agent used in this experiment was 20 µl. Another batch applied high concentration of GDP (100µl). And

finally, the last batch used 20 μ l of GDP but higher amount of GOx (12mg). Then, the sensor tip was dipped into the mixture and left to dry overnight.

After immobilizing GOD, the sensors tip were dipped into the PU (Tecoflex®, SG-60D)/THF solution twice to form a layer of diffusion-limiting membrane, which was allowed to dry completely. Figure 1 shows the constructed needle type sensor in reference to a wire and a five pence coin.

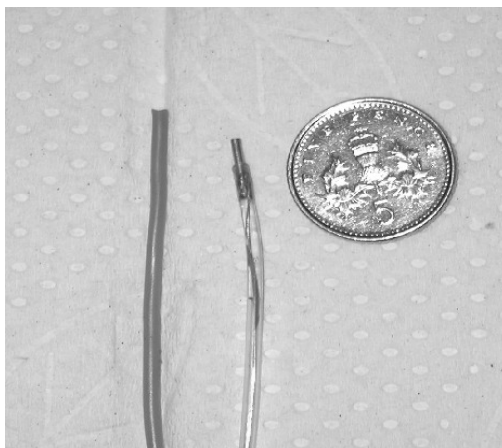


Figure 1 The constructed sensor (in the middle) compared to a wire and a 5 pence coin

2.2 Characterization of the Sensors

For every test, current generated in the sensors were measured based on amperometric principles. The potential applied between the working and the reference electrode was 650 mV. An amperometer (Lancaster University) with 0.01 nA resolution was used to record the current. All the measurements were performed at room temperature.

The glucose concentrations of the test solutions varied from 3.15 mM to 31.5 mM by adding concentrated glucose solution to the test solution. The fabricated glucose biosensors were dipped into the glucose solution and readings from the amperometer were recorded. The results were then compared.

3 0 Results

3.1 Sol gel technique with low amount of glucose oxidase

Figure 2 shows the relationship between glucose concentration and current measurement by biosensor utilizing 20 μ l of GDP and 2mg GOx. There was an increment of current until 9.45 mM of glucose concentration but then a steady decrease in measurement was recorded. The experiment was halted at glucose concentration of 22.05 mM since the result shows a further decrease in the current measurement.

3.2 Sol gel technique with high volume of cross link agent

The relationship between glucose concentration and current measurement by biosensor with high concentration of GDP is depicted in Figure 3. The result shows that the sensor is able to

detect particular glucose concentrations and a linear response with a high correlation was observed. Therefore, the composition is considered sufficient to provide good and reliable results.

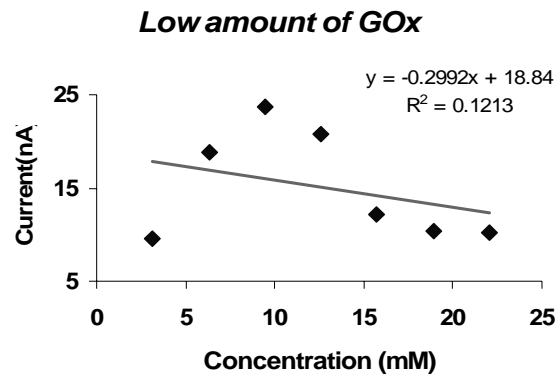


Figure 2 Glucose concentration versus current measurement by biosensor using sol gel method with low amount of glucose oxidase (20 μ l of GDP and 2mg GOx)

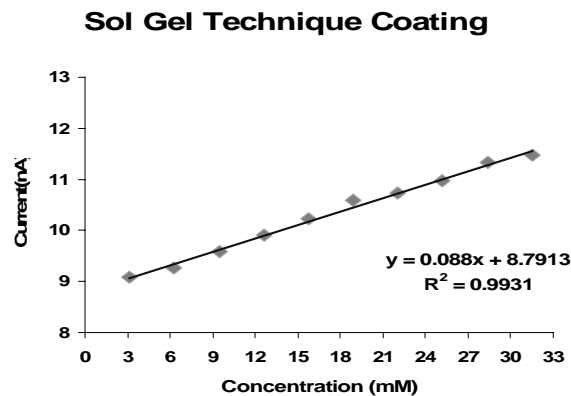


Figure 3 Glucose concentration versus current measurement by biosensor using sol gel method utilizing high volume of cross link agent

3.3 Sol gel technique with high amount of glucose oxidase

As shown in Figure 4, maximum current is observed at 6.3 mM of glucose concentration and then the current measurements continue to decrease steadily. The experiment was ended after few readings since the results are unable to show positive linear measurement of a typical glucose oxidase biosensor.

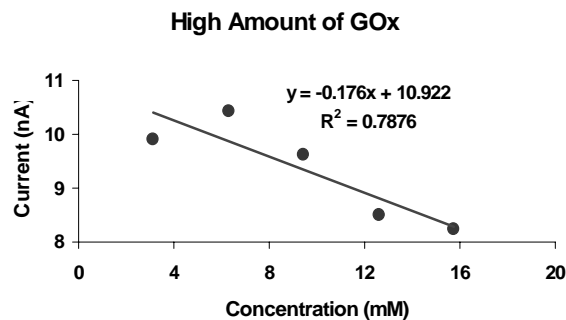


Figure 4 Glucose concentration versus current measurement by biosensor using sol gel method with high amount of glucose oxidase (20 μ l of GDP mixed with high amount of glucose oxidase)

4.0 Discussions

Figure 2 shows maximum current measurement is observed at 9.45 mM. After that, the current measurement showed a steady decrease. The situation might occur due to saturation of the analytes. However, the amount of enzyme was low and the enzyme used was not enough to react with the glucose. In this case, the cross link agent could not assist in obtaining a better result because the enzyme used was insufficient. Thus, the appropriate amount of glucose oxidase is another important factor to be considered in designing a biosensor.

The composition used later was a combination of high amount of glucose oxidase and high concentration of cross link agent. Unlike previous composition, this composition was able to produce superior results and this particular sensor could detect low concentration of glucose. The sensor applying this technique could detect glucose and the current produced was proportional to the glucose concentration. A linear response with high correlation had been obtained from the measurement. For this composition, the amount of enzyme was sufficient for glucose detection. The cross link agent was adequate to retain the enzyme to the electrode and no leaching happened. This resulted in steady increases in current measurement in response of glucose addition.

The last composition used high amount of glucose oxidase, twice than the one used in previous composition. As shown in Figure 4, the steady increase of current was achieved up to 6.3 mM of glucose concentration. The amount of glucose oxidase applied was high compared with the previous composition. However, the results were still poor. This is most probably due to the insufficient amount of cross link agent that could retain the enzyme to the electrode. Although the amount of glucose oxidase was high but the sensor was still not reliable enough to detect glucose proportionally to the amount of glucose added. Enzyme leaching might be the most suitable explanation to this incident which results in the decrease of measurement after certain time thereafter. Therefore, the amount of cross link agent plays a significant role in determining the success of sol gel technique rather than the amount of enzyme used.

5.0 Conclusions

The results obtained in this project had confirmed that the right amount of cross link agent and the glucose oxidase used in the sol gel technique is important in producing a reliable biosensor. In this study, the best composition for sol gel technique is to utilize high amount of cross link (20 μ l of GDP) and sufficient amount of glucose oxidase (6mg of GOx) in a neutral pH. The right composition gives positive linear response with high correlation.

Suitable amount of cross link agent is desired to bind the enzyme to the silicate. If the cross link agent is insufficient, the leaching of the enzyme would occur, and the sensor will fail to measure the glucose concentration correctly. The sensor will be able to sense the analyte at certain concentration and then the current readings will start to decrease. This type of observation defeats the purpose of linear measurement of a typical glucose oxidase biosensor.

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